# REMARKS

## The Present Invention

The present invention is directed to a universal bystander cell line, which is a human cell line, naturally lacks major histocompatibility class I (MHC-I) antigens and major histocompatibility class II (MHC-II) antigens, and is modified by introduction of a nucleic acid molecule comprising a nucleic acid sequence encoding granulocyte macrophage-colony stimulating factor (GM-CSF) operably linked to a promoter. The universal bystander cell line expresses at least 500 ng GM-CSF/$10^6$ cells/24 hours. The present invention is also directed to a composition comprising the universal bystander cell line and a cancer antigen, a method of making the universal bystander cell line, a method of stimulating an immune response to a cancer in a human patient by administering to the patient the composition, which has been irradiated, and a method of cancer immunotherapy, in which the improvement comprises administering to a human patient having cancer the irradiated composition.

## The Pending Claims

Claims 1-14, 17-28, 40-47 and 50-53 are currently pending. Claims 1-14 are directed to the universal bystander cell line, whereas claims 17-21 are directed to the composition comprising the universal bystander cell line and a cancer antigen, claims 22-28 are directed to the method of making the universal bystander cell line, claims 40-47 are directed to the method of stimulating an immune response, and claims 50-53 are directed to the improved method of cancer immunotherapy.

## The Office Action

The Office has rejected all of the pending claims under 35 U.S.C. § 112, first paragraph, for alleged lack of description and alleged lack of enablement. Claims 1-14, 17-22, 26, 27, 40-47 and 50-53 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 1, 5, 7, 17, 20, 22, 28, 40, 41, 44, 45, 50 and 52 have been rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of and, therefore, unpatentable over Dranoff et al. in view of Ferrone et al. as evidenced by Thomas et al., whereas claims 1, 5, 7, 11, 17, 20, 22-24, 28, 40, 41, 44, 45, 50 and 52 have been rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of and, therefore, unpatentable over Dranoff et al. and Ferrone et al. in view of Shepard et al. or Polack et al. Claims 1-14, 17-28, 40-47 and 50-53 have been rejected under the judicially created doctrine of obviousness-type double patenting as unpatentable over claims 1-21 of U.S. Patent No. 6,464,973. Reconsideration is hereby requested.

Discussion of Rejections under Section 112, first paragraph

The Office has rejected all of the pending claims for alleged lack of description. This rejection is traversed for the reasons set forth below.

The Office contends that the specification fails to define the term "naturally." Yet, the specification makes it clear that "naturally" is distinguished from "modified." See, for example, the specification at page 5, lines 3-6, wherein the universal bystander cell line is described as a human cell line, which either "naturally lacks" MHC-I antigens and MHC-II antigens, or is "modified" so that it lacks MHC-I antigens and MHC-II antigens. What is meant by "modified" is evidenced in the specification at, for example, page 7, lines 12-14, wherein it is stated that cells that lack MHC-I antigens can be achieved by interfering with the expression and/or transport of the $\alpha$ chain, whereas cells that lack MHC-II antigens can be achieved by interfering with the expression and/or transport of the $\alpha$ and $\beta$ chains. Thus, a cell line that has been modified so that it lacks MHC-I and MHC-II antigens is one that has been manipulated by man so as to lack MHC-I and MHC-II antigens. By contrast, a cell line that naturally lacks MHC-I and MHC-II antigens is one that lacks MHC-I and MHC-II antigens without manipulation by man, i.e., it never had MHC-I and MHC-II antigens or, as a result of the effects of nature, it came to lack MHC-I and MHC-II antigens. This is supported by the Office's reference to a standard English dictionary as evidencing that the term "naturally" means "by nature, inherently," "without a doubt," and "present or produced by nature" (see Office Action at page 3, third full paragraph) and the Office's acknowledgement that "naturally" encompasses cells, which have lost the capacity to express MHC antigens as a result of naturally occurring mutations, as supported by the references previously provided by Applicants (see Office Action at page 4, first full paragraph).

For the Office to go on to characterize the loss of MHC antigen expression as a result of a cancerous mutation as not "naturally" occurring at the top of page 5 of the Office Action is directly contrary to the evidence of record, including the evidence entered into the record by the Office, itself, by way of the instant Office Action. It is Applicants' position that this is the only and, hence, ill-founded basis upon which the Office can continue to maintain a rejection for lack of written description.

In this regard, the Office's reliance on the written description guidelines does nothing to support its position. The section of the written description guidelines to which the Office points is directed to a genus in which the species vary widely. In the case at hand, the species of the genus are all characterized by the same trait, the natural lack of MHC-I and MHC-II antigens.

The Office's citation of *Amgen* also does not support its position. The quote from *Amgen* upon which the Office relies is directed to chemical compounds, which are defined by

biological effect, rather than how to distinguish the chemical compounds from other materials. In contrast, the universal bystander line is characterized by (i) being a human cell line, (ii) naturally lacking MHC-I and MHC-II antigens, and (iii) being modified to express 500 ng or greater GM-CSF/$10^6$ cells/24 hrs. These are ALL characteristics of the cell line, itself.

In the event the Office is not persuaded by the above, Applicants request that the Office suggest alternative language. Perhaps, "unmodified" or "not modified" would suffice.

The Office also points to "defined" with regard to culture medium as lacking written description. Yet, "defined" is indicated to mean serum-free at page 14, line 26, of the instant specification as appreciated by the Office at page 7 of the instant Office Action. It is the presence of serum that renders a culture medium undefined inasmuch as the components of serum are unknown or undefined. A defined culture medium, by contrast, is one for which the chemical composition and the concentration of each and every component are known. See, for example, "Media Formulations" on page 2 of enclosed "Media for Tissue Culture."

While the Office points to Winchester et al. as allegedly evidencing that the SK-MEL-33 cells of Wang et al. do not lack MHC-II expression, this can be explained by differences between subclones, for example. Even if, for the sake of argument, the SK-MEL-33 cells express MHC-II, this does not detract from the other examples of cell lines, which lack MHC-I and MHC-II expression, provided by Applicants. In this regard, Applicants point out that numerous other examples of cell lines that lack MHC-I and MHC-II expression can be identified by searching the PubMed database for "HLA loss and tumors." Furthermore, Applicants need not describe that which is known in the art.

For the above reasons, Applicants submit that the claims do not lack description. Accordingly, Applicants request the withdrawal of this rejection.

The Office has rejected all of the pending claims for alleged lack of enablement. This rejection is traversed for the reasons set forth below.

According to the Office, the specification does not teach which cells or cell lines naturally lack MHC-I and MHC-II, alone or in further combination with other elements as recited in the claims, nor the source of obtaining such cell lines. As indicated above, whether or not a given human cell line naturally lacks MHC-I and MHC-II antigens is readily ascertainable -- either the human cell line expresses MHC-I and/or MHC-II or it does not. In fact, the antibodies described in Example 1 of the instant specification can be used to make such a determination as taught by Example 1. While the Office contends that it would be undue experimentation for the ordinarily skilled artisan to determine whether or not a particular cell line lacks MHC-I and MHC-II antigens, alone or in further combination with B-lymphocyte markers, an EBV genome, and EBV-associated antigen, and a receptor for EBV, Applicants point out that routine screening, even if on a "large-scale" basis, does **not**

constitute undue experimentation. See, e.g., *In re* Wands, 858 F.2d 731, 736-737 (Fed. Cir. 1988). The test for undue experimentation is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine. *See id.* at 737. Furthermore, as previously pointed out, numerous examples of such cell lines were known in the art prior to February 2, 1998, the date to which the instant application claims priority. See, e.g., SK-MEL-33 (Wang et al., J. Clin. Invest. 91: 684-692 (1993)), and other melanoma (Ferrone et al., Immunol. Today 16 (10): 487-494 (1995); Kageshita et al., Cancer Res. 53 (14): 3349-3354 (1993); and Wang et al., Tissue Antigens 47 (5): 382-390 (1996); abstracts attached) and cervical cancer cell lines. It is well-established that Applicants need not teach that which is known in the art.

The Office also points to "defined" with regard to culture medium as lacking enablement. Applicants respectfully submit that they need not enable that which is known in the art. As indicated above, the instant specification teaches that "defined" means serum-free at page 14, line 26. It is the presence of serum that renders a culture medium undefined inasmuch as the components of serum are unknown or undefined. By contrast, a defined culture medium is one for which the chemical composition and the concentration of each and every component are known. This is a basic principle of cell/tissue culture known to those of ordinary skill in the art as evidence by the enclosed "Media for Tissue Culture" at page 2, under "Media Formulations. "

The alleged discrepancy between Wang et al. and Winchester et al. has been addressed above. The alleged difference in MHC-II expression by SK-MEL-33 cells observed by Wang et al. and Winchester et al. can be due to differences between subclones, for example. Even if, for the sake of argument, the SK-MEL-33 cells express MHC-II, this does not detract from the other examples of MHC-I- and MHC-II-negative cell lines provided by Applicants. Also, as previously pointed out, numerous other examples of such cell lines can be identified by searching PubMed for "HLA loss and tumors."

In view of the foregoing, Applicants submit that the claims are enabled. Therefore, Applicants request the withdrawal of this rejection.

Discussion of Rejection under Section 112, second paragraph

Claims 1-14, 17-22, 26, 27, 40-47 and 50-53 have been rejected under Section 112, second paragraph, as allegedly indefinite. This rejection is traversed for the reasons set forth below.

The Office contends that the rejected claims are indefinite for recitation of "naturally." Yet, the definitions provided by the Office evidence that the term "naturally" means in a natural manner, by nature or natural means, and spontaneously. It is clear that the term encompasses the absence of MHC due to any natural cause, including a naturally occurring

mutation. What is excluded by use of the term "naturally" is the absence of MHC due to intervention by man, such as by manipulation of a cell to render it lacking in MHC.

The Office further contends that it is unclear whether or not all of the elements recited in claim 2 are required due to the use of the word "and" twice. The word "and" is used twice because, in the first instance, the nuclear antigen is that which is associated with the EBV genome. Therefore, the use of "and" twice does not render the claim indefinite.

Regarding the Office's contention that "defined medium" is vague and indefinite, Applicants respectfully submit that such a term is understood by one of ordinary skill in the art as a culture medium for which the chemical composition and the concentration of each and every component are known, which is not the case when serum is present (see page 14, line 26, of the instant specification where "defined" is indicated to mean serum-free). See, for example, "Media Formulations" on page 2 of the enclosed "Media for Tissue Culture" and further discussion herein on pages 4 and 5.

In view of the above, Applicants submit that the claims are definite. Therefore, Applicants request the withdrawal of this rejection.

Discussion of Rejections under Section 103(a)

Claims 1, 5, 7, 17, 20, 22, 28, 40, 41, 44, 45, 50 and 52 have been rejected under Section 103(a) as obvious in view of and, therefore, unpatentable over Dranoff et al. in view of Ferrone et al. as allegedly evidenced by Thomas et al. This rejection is traversed for the reasons set forth below.

Dranoff et al. does not teach or suggest a universal bystander cell line as taught by the present invention. Dranoff et al. also does not teach or suggest a composition comprising a universal bystander cell line, a method of making a universal bystander cell line, and a method of stimulating an immune response to a cancer in a human patient by administering the composition comprising a universal bystander cell line. Dranoff et al. does not appreciate the importance of using a cell line that naturally lacks MHC-I and MHC-II as taught by the present invention.

The Office admits that it is unclear whether the B16 melanoma disclosed by Dranoff et al. expresses MHC-I or MHC-II. The Office relies on Ferrone et al. as disclosing that various percentages of primary melanoma, metastatic melanoma, and melanoma cell lines lack MHC-I and normally lack MHC-II. The Office concludes that it would have been obvious to modify the methods of Dranoff et al. by substituting the B16 melanoma with melanoma cells that lack MHC-I and MHC-II as disclosed by Ferrone et al. with a reasonable likelihood of success. While admittedly not relying on Thomas et al., the Office attempts to bolster its reliance on the combined disclosure of Dranoff et al. and Ferrone et al. by pointing to Thomas et al. as disclosing that B78H1, a variant cell line of the B16 melanoma disclosed

by Dranoff et al., lacks MHC-I. Thomas et al., however, teaches that the expression of an allogeneic MHC molecule by a vaccine cell can actually enhance the induction of systemic antitumor immunity (see abstract).

As stated above, the Office concludes that the standard for obviousness merely requires that one of ordinary skill in the art at the time the invention was made would have been motivated to modify the teachings in the art to make and use the claimed invention, and the prior art need not specifically suggest or teach such a modification. This standard is not the appropriate standard to determine obviousness, since the level of skill in the art must be qualified to provide the motivation to modify or combine the prior art to meet the claims. *See Al-Site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308 (Fed. Cir. 1999). In order to use the knowledge of the ordinarily skilled artisan as the motivating factor to modify the prior art, the Office must make a factual finding as to the specific understanding within the knowledge of the ordinarily skilled artisan that would have provided the motivation to modify the prior art, thereby rendering the claimed invention obvious. *See In re* Kotzab, 217 F.3d 1365 (Fed. Cir. 2000). The Office has not made such a finding.

The appropriate test to establish a *prima facie* case of obviousness demands that the Office satisfy three requirements: (1) the Office must identify some suggestion or motivation, either in the references relied upon or in the knowledge generally available in the art, to modify the references in such a way as to arrive at the invention claimed, (2) there must be a reasonable expectation of success, and (3) the prior art references relied upon must teach or suggest all of the elements of the claim. As stated above, the Office has not met this burden by simply asserting that it believes that one of ordinary skill in the art would have found the present invention obvious.

None of the cited references disclose a universal bystander cell line as taught by the present invention. The present invention teaches a cell line that naturally lacks MHC-I and MHC-II. As pointed out above, and admitted by the Office, Ferrone et al. alone, or together with Dranoff et al., do(es) not explicitly disclose or suggest that a melanoma cell line must lack MHC-II in addition to lacking MHC-I. Neither Ferrone et al. in combination with Dranoff et al., nor the art, itself, provide any incentive to deviate from the teachings in the prior art. Thus, the Office cannot rely on the references cited to establish *prima facie* obviousness. The references simply do not suggest or motivate one to modify the art to arrive at the claimed invention. It is not enough for the Office to assert that one ordinarily skilled in the art would have been motivated to modify the references; some greater showing of motivation is necessary to establish a *prima facie* case for obviousness. Furthermore, there is no basis in the references to establish a reasonable likelihood of success for the claimed invention. Finally, not all limitations of the present invention are explicitly recited in the prior art. Hence, it is Applicants' position that the Office has failed to establish that the ordinarily skilled artisan would have been motivated to

substitute a cell line that lacks MHC-I and MHC-II for a cell line that lacks MHC-I in the method of Dranoff et al. in the absence of an explicit teaching or suggestion of the benefit in doing so.

In view of the foregoing, Applicants submit that the claimed invention is not obvious in view of the cited references. Accordingly, Applicants request the withdrawal of this rejection.

The Office has rejected the same claims and, additionally, claims 11, 23 and 24, which are directed to the use of hygromycin resistance as a selectable marker, under Section 103(a) as obvious in view of and, therefore, unpatentable over Dranoff et al. and Ferrone et al. in view of Shepard et al. or Polack et al. This rejection is traversed for the reasons set forth below.

As indicated above, Dranoff et al. does not teach or suggest a universal bystander cell line as taught by the present invention. Dranoff et al. also does not teach or suggest a composition comprising a universal bystander cell line, a method of making a universal bystander cell line, and a method of stimulating an immune response to a cancer in a human patient by administering the composition comprising a universal bystander cell line. Dranoff et al. does not appreciate the importance of using a cell line that naturally lacks MHC-I and MHC-II as taught by the present invention. Ferrone et al. does not cure the deficiencies of Dranoff et al. for the reasons set forth above. The fact that Shepard et al. or Polack et al. may disclose the use of hygromycin resistance as a selectable marker is of no import then. Neither Shepard et al. nor Polack et al. cures the deficiencies of Dranoff et al. and Ferrone et al.

Therefore, the claimed invention cannot be said to be obvious in view of the cited references. Accordingly, the withdrawal of this rejection is respectfully requested.

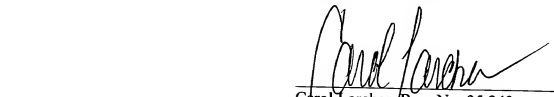
Discussion of Obviousness-Type Double-Patenting Rejection

The Office has rejected claims 1-14, 17-28, 40-47 and 50-53 under the judicially created doctrine of obviousness-type double-patenting as unpatentable over claims 1-21 of U.S. Pat. No. 6,464,973. Upon an indication of allowable subject matter, Applicants will submit a terminal disclaimer, which will render this rejection moot.

Conclusion

In view of the above, the application is considered to be in good and proper form for allowance, and the Office is respectfully requested to pass this application to issuance. If, in the opinion of the Office, a telephone conference would expedite prosecution, the Office is encouraged to contact the undersigned attorney.

Respectfully submitted,



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# Media for Tissue Culture

Factors affecting media:

**pH**

- Most cells grow well at pH 7.4
  - Some fibroblasts grow at 7.4-7.7
  - Transformed cells 7.0-7.4
  - Epidermal cells some times 5.5
- Phenol Red indicator used
  - Purple at pH 7.8
  - Pink at pH 7.6
  - Red at pH 7.4
  - Orange at pH 7.0
  - Yellow at pH 6.5
- $CO_2$ and Bicarbonate Buffering system

  must use buffers to prevent swings in pH

  related to equation catalyzed by carbonic anhydrase

  HEPES buffers are used but must also regulate $CO_2$ levels in incubator



**$O_2$ Levels**

- cells in culture rely mostly on Glycolysis
- rely on dissolved $O_2$
- too much leads to the introduction of free radicals

**Use Reducing Agents**

B-mercaptoethanol:

- Stimulate cysteine uptake by forming a mixed disulfide.
- Help prevent peroxide damage by restoring the reduced form of

glutathion.

**Osmolality**

- ensure proper osmotic tension for cells
- can tolerate certain range
- measures elevation of vapor pressure
- should check this as QC factor in media prep

**Temperature**

- maintain close to 37C
- better lower than over
- cells die at 40C
- tip: keep temp constant by getting in and out of incubators quickly

**Other Protective Agents**

Protect cells from damage by shear, Osmotic pressure, toxic metals and oxidative injury:

- Methyl cellulose
- Polyvinyl pyrolidine
- Dextran
- Pluronic F-68
- Polyethylene glycol
- Antioxidants

**Media Formulations**

Defined Medium: The chemical structure and the concentration of every component is known.

Undefined Medium: Contain one or more unidentified components.

## Basal Salt Mixtures

A mixture of:

- Calcium Chloride
- Magnesium Chloride
- Potassium Chloride
- Potassium Phosphate Monobasic (Anhydrous)
- Sodium Chloride
- Sodium Phosphate (Dibasic)

Functions of BSM:

- Provide water and certain bulk ions for cell metabolism
- Maintain intra and extracellular osmotic balance
- Provide a buffering system to maintain the medium within the physiological pH range

## Importance of Media Design

- Cell line stability
- Material requirement and storage
- Costs
- Product yield
- Product quality assurance
- Downstream processing
- Bioreactor design and operation

## The Most Common Types of Media

| | |
|---|---|
| Medium 199 | Morgan et.al., 1950 |
| MEM | Eagle 1959 |
| CMRL 1066 | Parker et.al., 1957 |
| DMEM | Dulbecco 1959 |
| Ham's F-12 | Ham 1965 |

| RPMI 1640 | Moore et.al., 1967 |
|---|---|

**Basic Components of Medium**

Water

Low Molecular weight nutrients

- Energy sources
- Nitrogen sources
- Vitamins
- Bulk Ions
- Lipids and Phospholipid precursors

Non-Nutrient substances

- Antibiotics
- Reducing agents
- Buffers
- DNA & RNA precursors
- Phenol Red
- Protective agents
- Attachment factors

**Water Quality**

Types of contaminants in water:

Inorganics: Heavy metals, iron, calcium, chlorine

Organics: Detergents or by-products of plant decay.

Bacterial products: Endotoxins:

- Lipopolysacharides generated by gram negative bacteria.

- Can be found in most biological products.

Use highly purified water for media preparations.

**Low Molecular weight Nutrients**

Energy Sources: Carbohydrates- Glucose

Nitrogen Sources: Amino Acids

**Essential amino acids:**

- Arginine
- Lysine
- Histidine
- Isoleucine
- Leucine
- Cystein
- Methionine
- Phenylalanine
- Threonine
- Tryptophan
- Tyrosine
- Valine
- Glutamine

**Non-essential amino acids:**

- Alanine
- Asparagine
- Asparatic acid
- Glutamic acid
- Glycine

- Proline
- Serine

Importance of Amino Acids

- Differentiation may lead to reduction in cell capacity to synthesize amino acids
- Biochemical specialization lost *in vitro*
- Failure to synthesize a particular AA in vitro may represent the lack of correct precursor or cofactor in artificial environment

  *Ex:* Monkey kidney cells have reduced ability to convert folic to folinic acid. The later is required as a cofactor for the conversion of Serine to Glycine

- Monolayer cells divide at a very active pace, thus insufficient amounts can be synthesized

**Importance of Glutamine**

- Support cell growth and AA uptake.
- Support glucose utilization.
- A source of Energy
- Support protein turnover

Decomposes in the medium in a time and temperature manner

| | |
|---|---|
| At 37C | half life = 8 days |
| At 4C | half life = 40 days |

*A product of decomposition is ammonia which is potentially toxic*

**Vitamins**

Water soluble:

- Biotin
- niacinamide
- Pyridoxine
- Thiamine

- Ascorbic Acid
- Folic Acid
- Riboflavin
- Vitamin B12
- Pantothenate

Fat soluble:

- Vitamin A
- Vitamin D
- Vitamin E
- Vitamin K
- E and K are toxic when in excess

**Bulk Ions**

Sodium: maintains osmotic pressure in the medium

Potassium: maintains osmotic pressure in the cell

Calcium and Magnesium:

- Essential for intracellular enzymes
- Participate Cell attachment and spreading
- Calcium is essential for cytoplasm progress

Iron: Needed for respiratory pigments-cytochromes

Carbonate:

- Natural buffering
- Participate in basic biochemical processes

Phosphate: energy carrier